

THE PROTECTIVE EFFECTS OF ALISKIREN ON LUNG HISTOPATHOLOGY AFTER
TRIOLEIN-INDUCED FAT EMBOLISM IN RATS

By

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Abstract

Background: Fat embolization (FE) and the consequent fat embolism syndrome (FES) remain poorly understood complications of skeletal and other major trauma. While FE and FES can lead to major pulmonary damage including ARDS and death, current treatment of FES is limited to supportive therapy. The renin angiotensin system (RAS) plays a significant role in the pathophysiology of FE, and drugs interfering with the RAS, captopril and losartan, have reduced histopathologic pulmonary damage in a rat model of FE. In this study, we examine the potential therapeutic effects of aliskiren, a direct renin inhibitor, on lung histopathology following FE.

Methods: A model of FE was created in unanesthetized rats using intravenous injection of the neutral fat triolein. Intraperitoneal injections of aliskiren at either 50 mg/kg or 100 mg/kg were performed one hour after FE induction via triolein. Rats were euthanized at 48 hours, and various pathology stains and methods were used to study and compare the lungs of these animals.

Results: The lungs of the triolein only treated animals showed severe gross and histopathologic damage which was mitigated by aliskiren. (1) Fibrosis: Rats treated with triolein alone showed significant fibrotic changes with increased collagen and myofibroblast activation ($p < 0.01$). Aliskiren blocked this inflammatory and profibrotic process to a level indistinguishable from the controls ($p < 0.01$). (2) Fat: Rats treated with triolein alone showed a statistically significant increase in fat ($p < 0.01$) with subsequent aliskiren administration at both doses reducing the size, distribution, and amount of fat droplets ($p < 0.01$). (3) Vasculitis: There was a trend in reduced lumen patency in the triolein only treated animals which improved after aliskiren treatment.

Conclusions: Aliskiren protected the lungs of these rats from FE-induced pulmonary damage at 48 hours. Clinical implications include the use of aliskiren both prophylactically (before certain orthopedic procedures) and therapeutically (after severe trauma) to prevent the consequent severe pulmonary fibrosis of FE.

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Introduction

Definitions and Relevance

Fat embolism (FE) is the presence of fat globules in the peripheral circulation and lung parenchyma and is primarily subclinical. Fat embolism syndrome (FES) is a serious consequence of FE, typically manifesting as distinct triad of pulmonary, dermatologic, and neurologic dysfunction. While majority (95%) of FES cases develop following major orthopedic trauma¹, a multitude of less common risk factors include orthopedic procedures, extensive burns, bone marrow harvest, diabetes mellitus, pancreatitis, liposuction, sickle cell crisis, alcoholic liver disease, and other soft tissue injury.²

FE leads to either negligible lung injury or, less frequently, to a cascade of events presenting as FES and serious acute respiratory distress syndrome (ARDS). While it was previously believed that there are no long-term pulmonary sequelae after subclinical FE or even clinical FES, it has recently been reported in a follow-up study on 5-year outcomes that survivors of ARDS do not return to normal predicted levels of physiological function.³ In addition, the lungs of animals exposed to FE demonstrate severe pulmonary fibrosis and heightened vulnerability to further exposure of an insult.⁴ Thus, while FE might not initially produce clinically obvious respiratory problems, there may be a silent inflammatory and fibrotic process occurring with harmful implications later in life such as contribution to the development of idiopathic pulmonary fibrosis.

Incidence and Mortality

FE has been estimated to occur in more than 90% of patients with traumatic injury.⁵ Further confirmation of this high incidence figure came from a recent autopsy series reporting fat emboli in the pulmonary circulation of 82% of trauma patient.⁶ FES has a wide range of

incidence rates in current orthopedic and trauma literature ranging from as low as 0.2-0.9% in retrospective studies and as high as 35% in prospective studies.¹ The great variability in incidence rates can be accounted for by the heterogeneity of diagnostic criteria, inconsistent clinical recognition, or overshadowing by other illnesses or injuries. Mortality figures are also discrepant with an overall mortality rate ranging from 2.5-20% as recently described in the current literature.¹ If FE is a risk factor for later development of IPF as we have proposed from suggestive findings, broader implications related to the burden of IPF include the 200,000 people in the US^{7, 8} with IPF contributing to 40,000 deaths each year and an estimated annual IPF-attributable medical cost of \$2 billion to the U.S. health care system.⁹

Treatment

To date, there are no pharmacologic therapies for the prevention or treatment of FES, which may contribute to the high mortality rates. Current treatment of FES is limited to supportive care including respiratory support (oxygen, mechanical ventilation), volume replacement for hemodynamic stability (fluids, blood products), and analgesia.¹⁰ Therapies such as heparin and corticosteroids¹¹⁻¹⁵ have been employed in humans, however, neither have reliably been proven to improve morbidity and mortality.

Experimental Models of FE

Since it is generally not feasible to study the pathophysiology of FE and FES in humans, much of our understanding is based on systematic experimentation in animals. The diversity of animal models of FE to simulate that of humans is broad including the displacement of the animals' bone marrow fat into circulation or direct, intravenous injection of a mimicking substance (neutral fats, fatty acids, or the animal's own subcutaneous fat).^{9, 16-19} Our model of FE developed and employed in past studies^{4, 20-22} and the current study has several advantages. First,

we use the neutral fat triolein, the predominant fat bone marrow,²³ rather than oleic acid, a toxic fatty acid thought to be produced by lipases in the lung.^{24, 25} Second, the rats are conscious during the intravenous injections of triolein, avoiding the complications of anesthesia on respiratory function.²⁶ Third, the experiments are carried out at 48 hours post-triolein injection based on our previous studies demonstrating peak histopathological effects at this time point.²⁰ Lastly, the same histopathological pulmonary damage as demonstrated in our animal model has been found in FES studied in human autopsies, strengthening the confidence for application to humans.²⁷

Proposed pathophysiology

The renin-angiotensin system (RAS) has been implicated in the pathophysiology of FE, notably through the pro-fibrotic properties of Angiotensin II (Ang II).²⁸ Inhibition of RAS through various mechanisms has proved to be a successful strategy for mitigating the histopathologic damage after FE.²¹ The protective effects of an angiotensin-converting enzyme (ACE) inhibitor, captopril, and an Ang II type I receptor blocker, losartan, underlined the involvement of Ang II and the Ang II type I receptor. However, since these past results did not indicate 100% blockade of FE-induced pathology, there is uncertainty whether this Ang II is solely a product of ACE or if there is involvement of another enzyme such as Ang II- forming chymase acting on angiotensin I (Ang I).²⁹ In addition, an increase in renin has been demonstrated in the lungs³⁰ and kidneys³¹ post-triolein injection, justifying the attempt to block renin as a mediator.

As an extension of our previous findings, we have employed the use of aliskiren, a direct renin inhibitor, in our FE model in rats. The use of aliskiren allows us to examine the effect of directly inhibiting the rate limiting enzyme, renin, in Ang I and ultimately Ang II production. In

this study, we investigate the effects of aliskiren on lung histopathology following triolein-induced FE to further elucidate the roles these various RAS mediators play in the pathogenesis of FE.

Materials and Methods

Responsible Conduct of Research

This study was conducted under the approval of the University of Missouri at Kansas City Institutional Animal Care and Use Committee (IACUC), Protocol # 1507. Animal care and procedures were in accordance with institutional guidelines.

Animals and Experimental Design

Following approval of the protocol, we obtained a total of 22 male Sprague-Dawley rats (280-300 g) from Harlan Laboratories (Indianapolis, IN) and divided them into four groups as presented in table 1. Pure triolein (glyceryl trioleate, Sigma grade; Sigma Corp., St. Louis, MO) was used to simulate a FE. Group A (n=4) controls received 0.2 mL intravenous (i.v.) normal saline (NS) at 0 hours and 0.2 mL intraperitoneal (i.p.) NS at hour 1. Group B (n=6) received pure triolein 0.2 mL i.v. at 0 hours and NS 0.2 mL i.p. at hour 1. Group C (n=6) received the same dose of triolein at 0 hours and aliskiren 50 mg/kg at hour 1. Group D (n=6) received the same dose of triolein at 0 hours and aliskiren 100 mg/kg at hour 1.

The dose of triolein was selected based on our previous work with unanesthetized rats^{4, 20-22} which demonstrated this dose to induce very severe pathologic changes coupled with a low incidence of mortality. The doses of aliskiren have been used in rats in published experiments at different time periods: 50 mg/kg i.p. daily for up to 8 weeks³² and 100 mg/kg i.p. for only 60 minutes.³³ While aliskiren is orally active, we chose intraperitoneal injections of aliskiren for this study rather than oral administration to mimic our previous experiments. Intraperitoneal

administration avoids potential aspiration which would complicate our findings and the validity of our results when studying pulmonary histopathology. Similar to the previous experiment with captopril and losartan,²¹ aliskiren was used as a rescue drug 1 hour after the initial insult of triolein injection, more closely simulating the most common risk factor for FE, trauma. All rats were given libitum access to food and water, and clinical observations were made at various time points before and after the injections. No animals died early, and all 22 rats were euthanized at 48 hours using inhalation isoflurane.

Group	n	Day 0 Time 0 hours	Day 0 Time 1 hour	Day 2 Time 48 hours
A	4	Saline 0.2 mL i.v.	Saline 0.2 mL i.p.	Euthanasia
B	6	Triolein 0.2 mL i.v.	Saline 0.2 mL i.p.	Euthanasia
C	6	Triolein 0.2 mL i.v.	Aliskiren 50 mg/kg i.p.	Euthanasia
D	6	Triolein 0.2 mL i.v.	Aliskiren 100 mg/kg i.p.	Euthanasia

Table 1. Treatment Groups

Pathology Procedures

After euthanasia, rats were necropsied, and the viscera of the thoracic and abdominal cavity were removed and inspected for gross anatomic pathologic changes. The lower lobe of the right lungs were collected and placed in 10% buffered formalin solution for fixation. After 10 days of fixation, the specimens were paraffin embedded, cut in sagittal sections (4 μ m), and stained with hematoxylin and eosin (H&E) for histological evaluation and vessel measurements. Slides were also stained with Masson's trichrome (trichrome) for collagen identification. Immunolabeling of lung alpha-smooth muscle actin (α -SMA) as a marker for myofibroblasts activation and differentiation was performed using previously described protocols.³⁴ The lower lobe of the left lungs were frozen at -20°C, sectioned, and stained for fat using Oil Red O.

Histologic evaluation was performed by two researchers who were blinded to the slide identity. Histopathologic studies were done on slides stained for H&E, trichrome, α -SMA, and Oil Red O. Ten photographs of each H&E slide were taken at 400x magnification. From these photographs, the lumen patency and media to adventitia ratio of at least five small caliber pulmonary arteries and arterioles were measured per slide. The lumen patency of these vessels were evaluated by measuring the luminal diameter divided by the external diameter of the vessel media. The media-to-adventitia ratio of these vessels were evaluated by measuring the external diameter of the media divided by the external diameter of the adventitia. Figure 1 shows the graphic representation. Eight photographs of each Oil Red O stained slide were taken at 100x magnification, ten photographs of each trichrome stained slide were taken at 400x magnification, and ten photographs of each α -SMA stained slide were taken at 400x magnification. The slides stained with Oil Red O, trichrome, and α -SMA were then digitally analyzed using the Image J Software (NIH) to quantify the amount of fat, collagen, and myofibroblasts present in each slide, respectively. See figure 2 for Image J demonstration.

Statistical Analysis

Data was statistically analyzed using analyses of variance, and, where appropriate, comparisons were made using Fisher's least significant differences. The level of statistical significance for comparison was set at $p=0.01$. The data analysis for this study was generated using SAS software, Version 9.2 of the SAS System for the University of Kansas Medical Center.

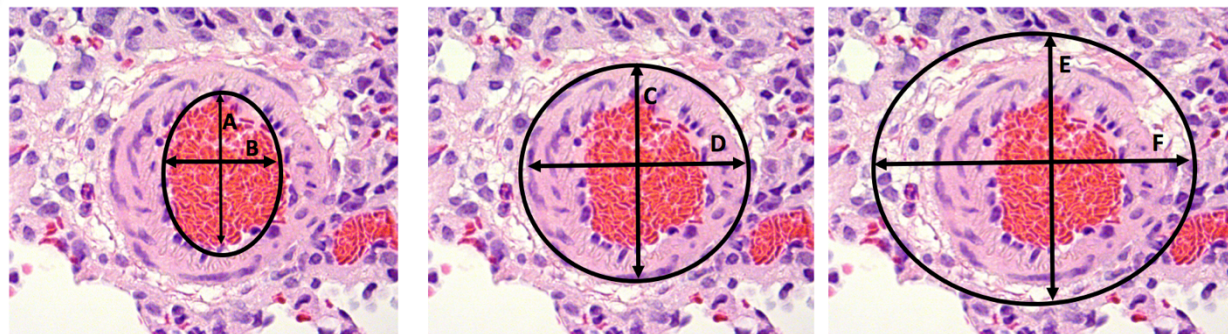


Figure 1. Graphic representation of the lumen patency and media-to-adventitia ratio measurements.

$$\text{Lumen Patency} = (A+B)/(C+D)$$

$$\text{Media-to-adventitia ratio} = (C+D)/(E+F)$$

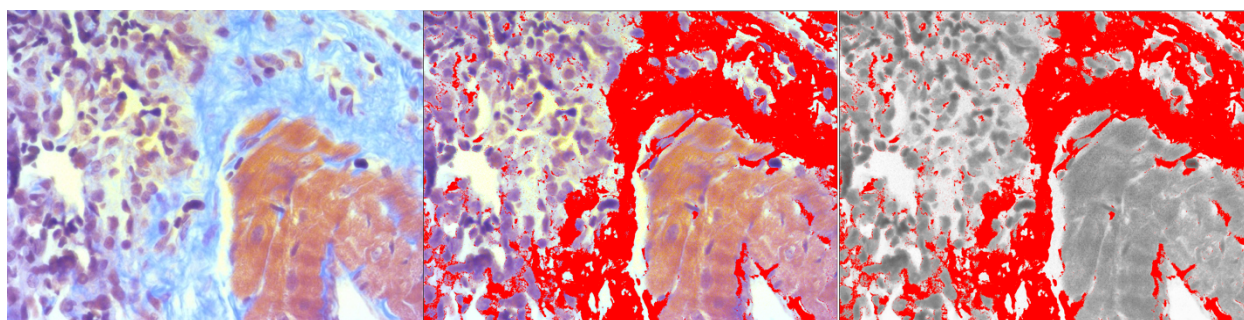


Figure 2. Graphic representation of Image J analysis of a trichrome-stained slide. The Image J software is capturing the amount of collagen (blue) as a percentage of the total area of the photograph. In this example, % area= 28.943.

Results

Behavior

All rats survived the injections. The rats injected with triolein became hypoactive immediately following the injections, but regained activity within a couple of hours and were indistinguishable from the controls as seen in our past studies.^{4, 20-22} On days 1 and 2, the rats continued to show no apparent signs of distress.

Gross Organ Appearance at Necropsy

The gross organ anatomy of the controls showed clean surfaces of the lungs with no pathology. Multiple organs of the triolein-injected rats in group B displayed gross pathologic

changes: liver congestion evidenced by a dark, speckled appearance; cardiovascular changes including right ventricular and inferior vena cava dilatation; and congestion and enlargement of the spleen. The lungs exhibited severe congestion as evidenced by diffuse pleural hemorrhages and cobblestoning of the pleural surface. The animals treated with aliskiren in groups C and D showed less pulmonary pathology with smooth pleural surfaces, minimal if any pleural hemorrhages, and less organ congestion. Two unique findings included an animal in group C with left renal deposits representing possible inflammation or calcification, and an animal in group D with notable ascites and organ pathology similar to the triolein-only treated animals.

Pulmonary Histopathology (H&E Staining)

Representative H&E stained slides from the four different treatment groups are presented in figure 3. Rats of the control group demonstrated patent small caliber arteries and arterioles with appropriately few inflammatory cells throughout pulmonary parenchyma. Histologic findings in the triolein-only treated animals, group B, mirrored that of past studies, confirming the reproduction of our FE model. Numerous signs of pathology were present in these animals: diffuse hemorrhages present in the thickened septa; congestion and diffuse inflammation comprised of neutrophils, lymphocytes, macrophages, and erythrocytes; and scattered areas of atelectasis and emphysema. Bronchial changes included thickening of the bronchial musculature, epithelial hypertrophy and inflammation, variable areas of disepithelialization, and loss of cilia. The lumen of large bronchi were also filled with edematous fluid, erythrocytes, and inflammatory cells. The small arteries and arterioles showed signs of severe vasculitis: thickened media, reduced luminal patency, and periadventitial edema. Some of these changes were present, but less severe, in the rats treated with aliskiren. Quantification of lumen patency and media-to-adventitia ratios are depicted in Figure 4. In the triolein- only treated animals, there was a trend

in reduced lumen patency compared to the controls, albeit not statistically significant, representing the vasculitis and media hypertrophy resulting after triolein injection ($p=0.0641$). Improvement in the aliskiren-treated animals was evidenced by increased lumen patency in the aliskiren 50 mg/kg group ($p=0.0058$), without statistical significance among the aliskiren 100 mg/kg group ($p=0.1134$). No difference was observed in the media-to-adventitia ratios among treatment groups.

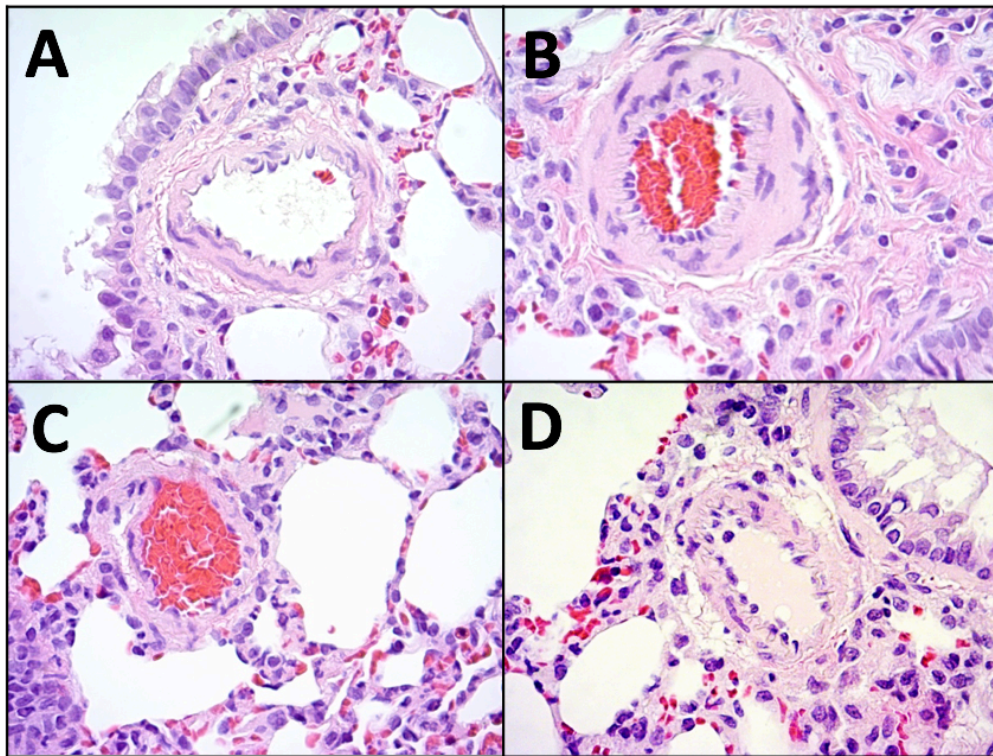


Figure 3. Representative hematoxylin and eosin (H&E) stained slides from the lungs of the four different treatment groups: saline + saline controls (A), triolein + saline (B), triolein + aliskiren 50 mg/kg (C), and triolein + aliskiren 100 mg/kg (D). Photographed at 400x. The triolein only group (B) demonstrated marked thickening of the arterial walls with severe septal inflammation. The histopathology improved with increased lumen patency of the arteries after aliskiren administration.

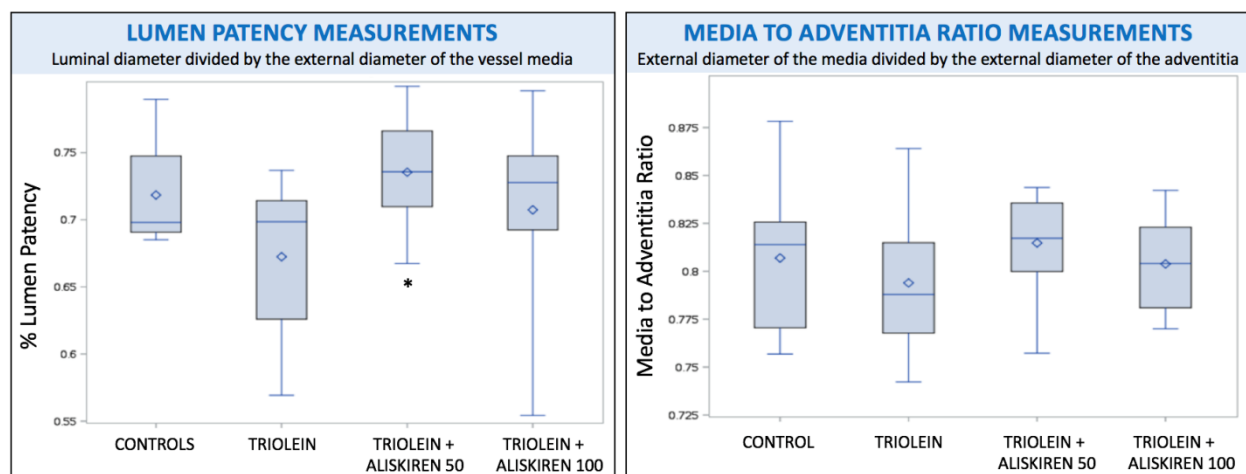


Figure 4. Effects of aliskiren on triolein-induced arterial lumen patency (A) and media-to-adventitia ratio (B). Asterisks indicate a significant difference from the triolein group, $p < 0.01$. While there is a trend of decreased lumen patency in the triolein-only group with respect to the controls, the number of vessels evaluated only allowed for detection of a significant difference between the two indicated treatment groups. No difference was observed in the media-to-adventitia ratios.

Pulmonary Histopathology (Trichrome and α -SMA Staining)

Representative trichrome and α -SMA stained slides from the four different treatment groups are presented in figures 5 and 6. In the triolein-only treated animals, trichrome staining showed collagen diffusely present in the parenchyma, media and adventitia of small-caliber arteries and arterioles, peribronchial musculature, and subpleural regions. The location and distribution of α -SMA expression in myofibroblasts closely paralleled that of the collagen. However, α -SMA staining was more intense and diffusely present in the intraseptal and perivascular areas. α -SMA expression and fibrosis were significantly reduced in both groups of the aliskiren-treated animals. Quantification of trichrome and α -SMA content by Image J confirmed that rats treated with triolein alone, group B, showed statistically significant fibrotic changes with increased collagen (Masson's trichrome) and myofibroblast activation (α -SMA) compared to the others three groups ($p < 0.01$) as seen in figures 7 and 8. Aliskiren blocked this inflammatory and fibrotic process by reducing the intensity of these stains to a level

indistinguishable from the controls ($p < 0.01$). Thus, there was no detectable difference in collagen or α -SMA expression between the controls and aliskiren treated animals at both doses.

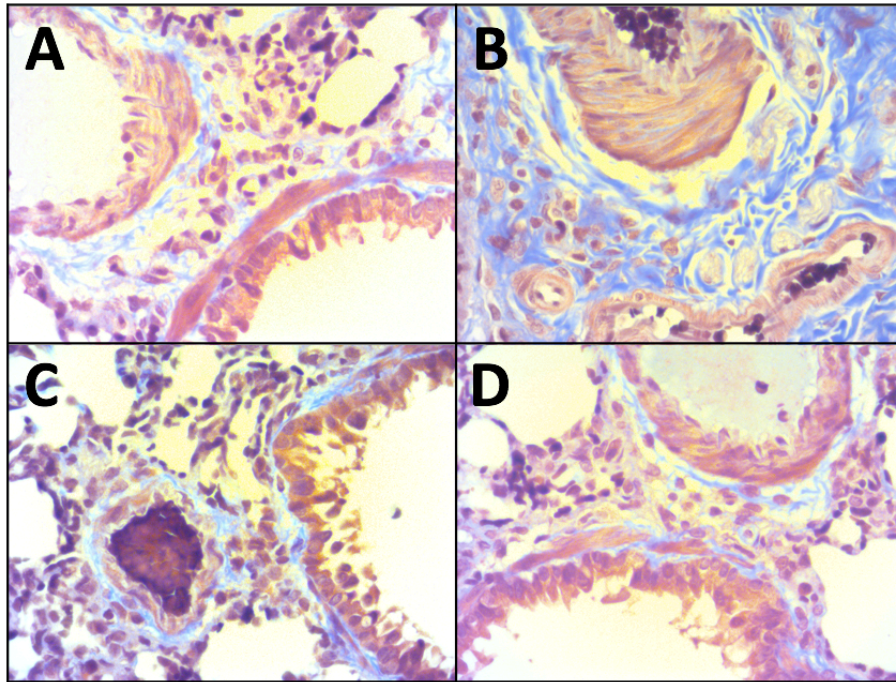


Figure 5. Representative trichrome-stained slides from the lungs of the four different treatment groups: saline + saline controls (A), triolein + saline (B), triolein + aliskiren 50 mg/kg (C), and triolein + aliskiren 100 mg/kg (D). Photographed at 400x. Collagen is stained blue. The triolein only group (B) demonstrates a severe inflammatory and fibrotic response evidenced by the increased collagen deposition in the perivascular and interstitial space. This response was blocked in the aliskiren-treated animals which displayed limited collagen mainly confined to the perivascular and peribronchial areas, similar to the controls (A).

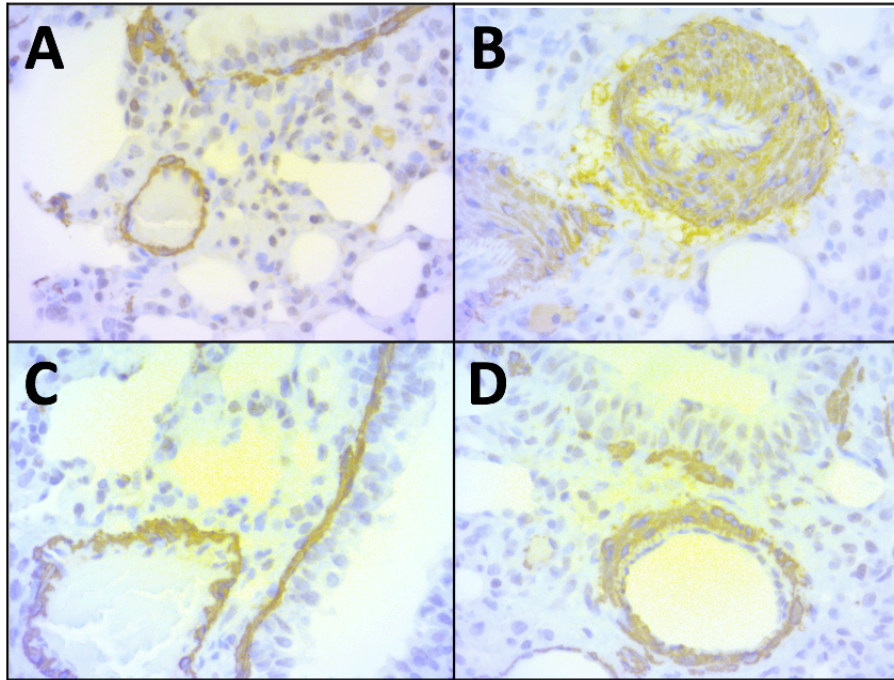


Figure 6. Representative alpha smooth muscle actin (α -SMA) stained slides from the lungs of the four different treatment groups: saline + saline controls (A), triolein + saline (B), triolein + aliskiren 50 mg/kg (C), and triolein + aliskiren 100 mg/kg (D). Photographed at 400x. Immunolabeling for α -SMA is represented in yellow-brown staining as a marker of myofibroblast activation and differentiation. Similar to the trichrome stain, the triolein only group (B) demonstrates a severe inflammatory and fibrotic response which was blocked in the aliskiren-treated animals.

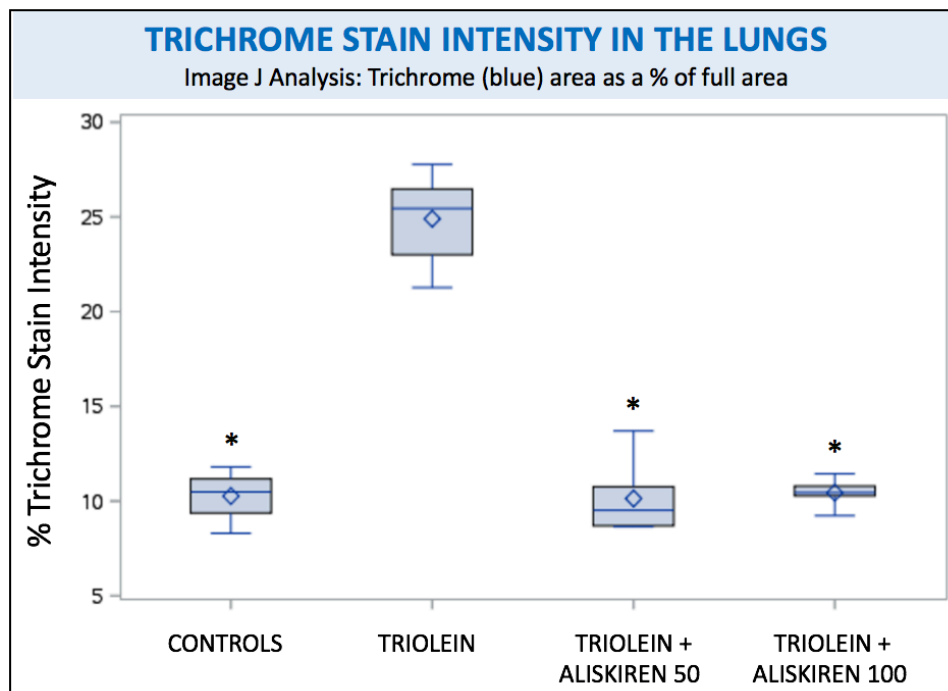


Figure 7. Image J analysis of the intensity of trichrome staining of rat lungs subjected to different treatments. Asterisks indicate significant difference from the triolein group, $p < 0.01$.

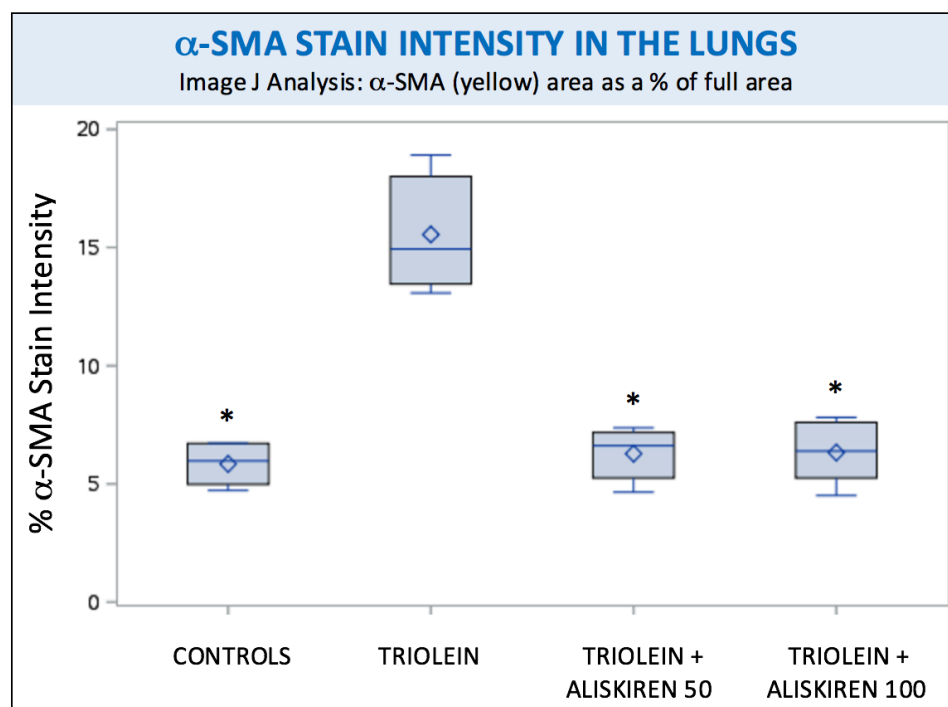


Figure 8. Image J analysis of the intensity of α -SMA staining of rat lungs subjected to different treatments. Asterisks indicate significant difference from the triolein group, $p < 0.01$.

Fat Distribution in Lungs (Oil Red O Staining)

Representative Oil Red O stained slides from the four different treatment groups are presented in figure 9. Saline injected controls had a minimal amount of fat present while the lungs of the triolein-only treated animals had a larger amount of fat which was present as small droplets diffuse throughout the pulmonary interstitial space or as large droplets around peribronchial veins; both groups confirm findings of our previous studies conducted at the same interval of time.^{20, 21} Fat droplets present in the rats treated with aliskiren were comparatively small in size and mostly located around peribronchial veins, traversing the vascular musculature. Few droplets were present throughout the alveolar septa. Rats treated with triolein alone, group B, showed a statistically significant increase in fat compared to the others three groups ($p < 0.01$). Aliskiren administration at both doses reduced the presence of fat to a level indistinguishable from the controls. (Figure 10). Thus, while a large amount of fat is present in the rat lungs after triolein administration, subsequent treatment with aliskiren results in a decreased amount of fat to a level with no significant difference from the controls.

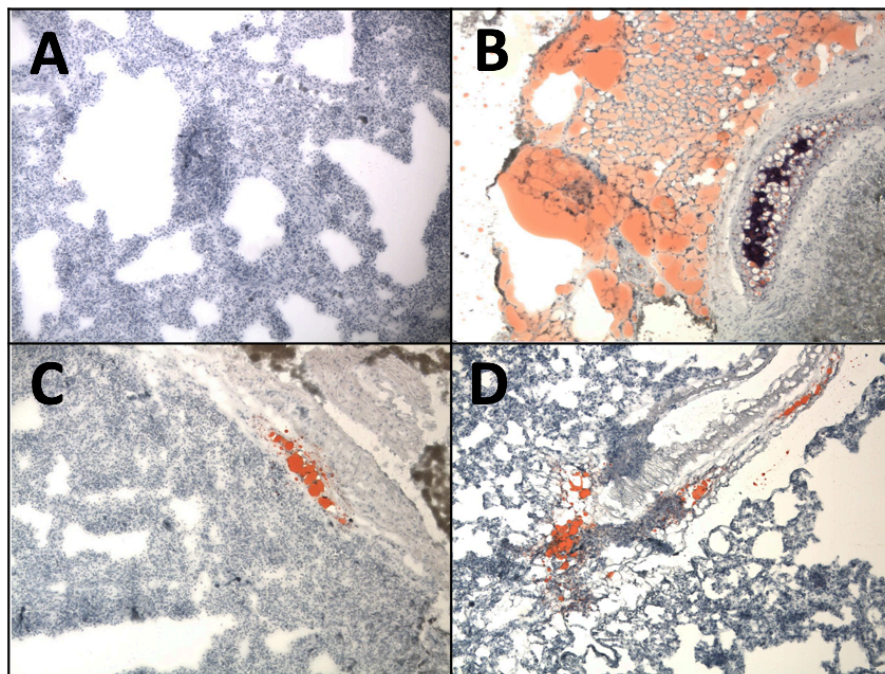


Figure 9. Representative Oil Red O stained slides from the lungs of the four different treatment groups: saline + saline controls (A), triolein + saline (B), triolein + aliskiren 50 mg/kg (C), and triolein + aliskiren 100 mg/kg (D). Photographed at 100x. Fat is stained red. The triolein only group demonstrated large fat globules diffuse through the pulmonary parenchyma. Aliskiren treatment resulted in comparatively smaller sized droplets with limited distribution confined to the perivascular areas.

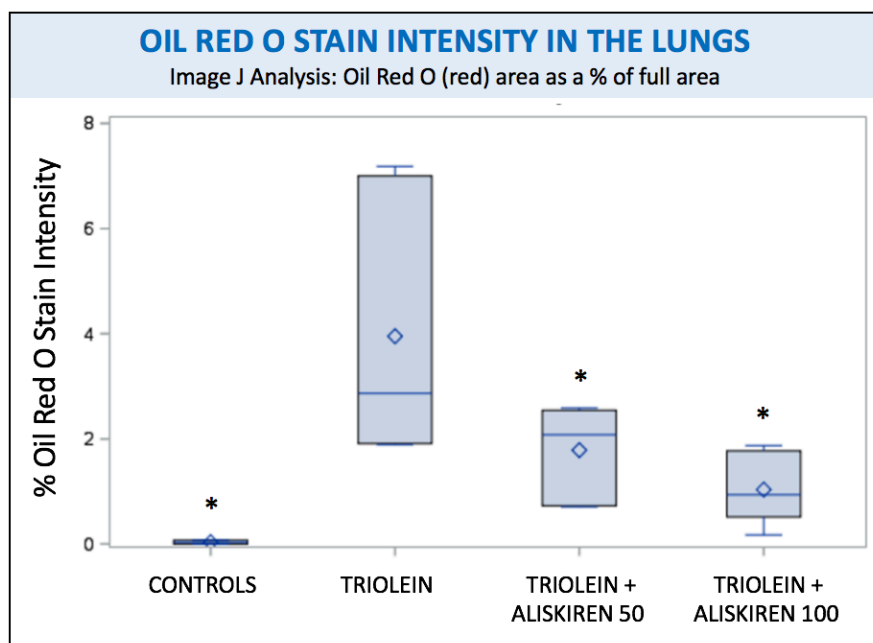


Figure 10. Image J analysis of the intensity of Oil Red O staining of rat lungs subjected to different treatments. Asterisks indicate significant difference from the triolein group, $p < 0.01$.

Discussion

Summary of Findings

These results extend the evidence of the central involvement of the RAS in the pathology of FE and the ability of a direct renin inhibitor, aliskiren, to mitigate this damage. In this study, renin inhibition via aliskiren resulted in attenuation of numerous pathologic changes found at 48 hours post- triolein administration. Some of these changes included (1) reduction in inflammation and pulmonary fibrosis as evidenced by the decrease in fibrotic marker expression (collagen and α -SMA) in the aliskiren treated animals compared to the triolein only group ($p<0.01$) (2) decreased presence and limited distribution of the fat in aliskiren treated animals compared to the triolein only group ($p<0.01$) which may be related to faster elimination of the injected triolein or to intracellular processes related to the RAS, and (3) improvement in vascular pathology and vasoconstriction as evidenced by a trend of increased lumen patency.

Pathophysiology of FE and relation to RAS

Among many, one of the currently accepted theories of FE maintains a three step progression: a mechanical phase begins at the site of insult with intravasation of fat into venous circulation and sequestration of this fat in the lung³; a latent phase of apparent resolution; and a chemical phase resulting from the action of pulmonary lipase which hydrolyzes the embolized neutral fats into more toxic free fatty acids,^{24, 25, 35} stimulating the release of inflammatory mediators. While much remains unknown, it has been demonstrated that many of these inflammatory mediators involve the RAS. Gonzalez et al. have suggested the cross-talk between pulmonary macrophages and mast cells as playing a key role in the activation of the local RAS.³⁶ They suggest that, in the presence of hypoxia, an intermediary substance released by alveolar macrophages stimulates peripheral mast cell production of renin.^{36, 37} Activation of the local

RAS ensues leading to the generation of pro-fibrotic Ang II and a general inflammatory response. Thus, the presence of fat globules in the lung may lead to macrophage activation, beginning the aforementioned cascade of events. Further support of this view comes from preliminary experiments that demonstrate an increased histochemical expression of angiotensin in the lungs²² and renin in the lungs³⁰ and kidneys³¹ of rats post triolein- induced FE.

Besides its classical role in blood pressure regulation, it has been previously shown that the RAS exerts pro-inflammatory effects, mainly through the action of angiotensin II (Ang II).²⁸ Ang II, generated by activation of the local RAS, plays a role in tissue repair and remodeling via Ang II's action on transforming growth factor beta (TGF- β), a potent inducer of fibroblast procollagen synthesis.³⁸ Through this mechanism, it is believed that Ang II stimulates fibroblast procollagen synthesis and promotes lung collagen deposition after lung injury. Ang II has also been demonstrated to increase vessel vasoconstriction, vascular permeability,²⁶ and lipase expression in vascular tissues³⁹ which would further increase the breakdown of triolein into more toxic fatty acids.

RAS Involvement in Other Models of Pulmonary Fibrosis

These pro-inflammatory effects of the RAS have been demonstrated to play an important role in multiple experimental models pulmonary fibrogenesis beyond FE. The lung histopathology found in this study is very similar to that found in other models of pulmonary injury and fibrosis where Ang II has been implicated as playing a key role in the pathogenesis. Agents interfering with the RAS have been found to abrogate experimental lung injury including the prevention of fibrotic, vascular, and bronchial changes induced by a wide variety of insults: bleomycin, hypoxia, radiation, monocrotaline, and others.⁴⁰⁻⁴⁸ This protective effect seems to be present whether the initial insult is a chemical, physical, or biological stimulus.

Potential Protective Properties of Aliskiren

We previously demonstrated the mitigating effects of captopril and losartan on lung histopathology, further elucidating this relationship between RAS and FE pathology.²¹ In the current study, we used aliskiren which also exerts its mechanism of action on the RAS, but through direct renin inhibition. It is important to note that, while many RAS-interfering drugs have been well studied in the different models of pulmonary fibrosis, there is limited research on the effects of aliskiren. To our knowledge, aliskiren has only been employed in two models of pulmonary fibrosis,^{48, 49} neither of which study FE.

Many properties of aliskiren and its mechanism of action may contribute to its protective effects presented in this study. Aliskiren effectively reduces functional plasma renin activity by binding to renin with high affinity and preventing it from converting angiotensinogen to Ang I. By inhibiting the rate-limiting step of Ang II formation, aliskiren produces more effective and complete inhibition of angiotensin II.⁵⁰ Aliskiren has also been suggested to inhibit local cardiac RAS in a rat model⁵¹ which may be extrapolated to other local RAS such as pulmonary RAS. In addition, recent studies indicate that aliskiren could be a potent inhibitor of the free forms of mature renin and of the receptor-bound forms of renin and prorenin,^{52, 53} and therefore aliskiren may offer pulmonary protection through its inhibition of both of these mediators.

There are some possible caveats that may explain aliskiren's partial, not complete, blockade of the triolein-induced histopathologic damage. First, both renin and prorenin maintain angiotensin-independent pro-fibrotic properties,^{54, 55} which are not blocked by aliskiren. Second, by inhibiting the production of Ang II, aliskiren also inhibits the production of anti-fibrotic Ang-(1-7), a breakdown product of Ang II.⁵⁶ Such implications include the possibility that a higher dose of aliskiren may block the protective effect of Ang-(1-7). Lastly, further possibilities

include incomplete inhibition of renin by aliskiren and/or the action of chymase or ACE on residual Ang I from plasma or tissue sources. However, the results distinctly indicate that renin is a major mediator of the RAS in the histopathological effects of FE in the rat.

Limitations

One limitation present in our study is the small sample size. The limited number of animals may have impacted the results of our vessel measurements including the non-statistically significant trend of decreased lumen patency among the triolein-only treated rats which we have found significant in multiple past FE studies.^{4, 20-22} In addition, the optimal dose, timing, and route of administration for aliskiren are yet to be defined. The optimal dose may be better identified in the future by performing a full dose response curve with aliskiren in these rats. Another potential approach is to pre-treat the rats with aliskiren prior to FE-induction to replicate clinical prophylactic treatment before certain orthopedic procedures. It is possible that pre-treatment may demonstrate a more complete blockade of the triolein-induced pathology. Lastly, the i.p. dosing of aliskiren selected in this study may complicate future translation into human subjects in which the approved route of administration is oral.

Future Research

Though there is much evidence authenticating the critical role of the RAS in FE and potential therapeutic modalities, many questions remain unanswered. Since other organs experience pathologic changes after FE which were protected by losartan,^{31, 57} the histopathology of the hearts and kidneys from these aliskiren-treated animals are currently under investigation. It would be desirable to do a dose response curve to determine which dosing regimen (dose, route, time) of aliskiren is most appropriate and effective. This study employed post-treatment with aliskiren at 1h post-insult to simulate the treatment in a trauma patient; however, pre-treatment

with aliskiren, which may be administered as prophylaxis before certain orthopedic procedures, may yield superior results. In addition, examination at later times beyond 48 hours could provide support that this protection blocks both acute and chronic changes observed in FE models.

Based on our current findings, avenues for future studies proposed by our group in rats subjected to FE include comparison of mast cell, renin, and prorenin staining amount and localization; measuring angiotensin peptides in lung tissue to differentiate angiotensin-independent effects of renin and prorenin; mast cell depletion or stabilization; macrophage depletion or stabilization; and Ang-(1-7) agonists and antagonists. By better defining the role of these the individual mediators of the RAS, targeted therapies such as aliskiren can be employed to protect the lungs and other organ systems from the resulting acute and chronic damage.

Clinical Implications

In summary, aliskiren protected the lungs of these rats from FE-induced pulmonary damage at 48 hours. These results provide further confirmation of the relationship between the RAS and FE pathology, and that drugs acting on different targets within the RAS may provide effective and targeted therapy for FES. Current treatment of FES is limited to supportive therapy with mortality figures as high as 20%.¹ Since it is suggested that both subclinical FE and clinical FES have long term consequences including ARDS, pulmonary fibrosis, and possible progression to IPF or COPD, identification of a specific therapy is warranted. Clinical implications include the use of aliskiren or other drugs acting on the RAS both prophylactically (before orthopedic procedures) and therapeutically (after severe trauma) for FE to prevent the consequent FES and severe pulmonary fibrosis.

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